Hum Genet (2003) 112 : 600–609
DOI 10.1007/s00439-003-0910-z

ORIGINAL INVESTIGATION

**Barbara Nemesure · Xiaodong Jiao · Qimei He
M. Cristina Leske · Suh-Yuh Wu · Anselm Hennis
Nancy Mendell · Joy Redman · Henri-Jean Garchon
Richa Agarwala · Alejandro A. Schäffer
Fielding Hejtmancik · Barbados Family Study Group**

A genome-wide scan for primary open-angle glaucoma (POAG): the Barbados Family Study of Open-Angle Glaucoma

Received: 31 July 2002 / Accepted: 4 January 2003 / Published online: 4 March 2003

B. Nemesure (✉) · Q. He · M. C. Leske · S.-Y. Wu · A. Hennis
B. F. S. Group
Department of Preventive Medicine,
School of Medicine, Stony Brook University,
Stony Brook, New York 11794–8036, USA
Tel.: +1-631-4441290, Fax: +1-631-4447525,
e-mail: bnemesure@notes.cc.sunysb.edu

X. Jiao · J. Redman · F. Hejtmancik · B. F. S. Group
Ophthalmic Genetics and Visual Function Branch,
National Eye Institute, National Institutes of Health,
Department of Health and Human Services,
Bethesda, Maryland, USA

A. Hennis
School of Clinical Medicine and Research,
University of the West Indies, Barbados, West Indies

A. Hennis · B. F. S. Group
Ministry of Health, Barbados, West Indies

N. Mendell
Department of Applied Mathematics and Statistics,
Stony Brook University, College of Arts and Sciences,
Stony Brook, New York, USA

H.-J. Garchon
INSERM Unit 25, Hôpital Necker, 161 Rue de Sèvres,
75743 Paris cedex 15, France

R. Agarwala
Information Engineering Branch,
National Center for Biotechnology Information,
National Institutes of Health, Department of Health
and Human Services, Bethesda, Maryland, USA

A. A. Schäffer
Computational Biology Branch,
National Center for Biotechnology Information,
National Institutes of Health, Department of Health
and Human Services, Bethesda, Maryland, USA

B. F. S. Group
The Johns Hopkins University School of Medicine,
Baltimore, Maryland, USA

Present address:
J. Redman
Quest Diagnostics, San Juan Capistrano, California, USA

Present address:
H.-J. Garchon
Universit Réné Descartes Paris V and Service d'Anatomie
Pathologique, Hôpital Européen Georges Pompidou, Paris, France

Abstract Primary open-angle glaucoma (POAG) is characterized by damage to the optic nerve with associated loss of vision. Six named genetic loci have been identified as contributing to POAG susceptibility by genetic linkage analysis of mostly Caucasian families, and two of the six causative genes have been identified. The Barbados Family Study of Open-Angle Glaucoma (BFSG) was designed to evaluate the genetic component of POAG in a population of African descent. A genome-wide scan was performed on 1327 individuals from 146 families in Barbados, West Indies. Linkage results were based on models and parameter estimates derived from a segregation analysis of these families, and on model-free analyses. Two-point LOD scores >1.0 were identified on chromosomes 1, 2, 9, 10, 11, and 14, with increased multipoint LOD scores being found on chromosomes 2, 10, and 14. Fine mapping was subsequently carried out and indicated that POAG may be linked to intervals on chromosome 2q between D2S2188 and D2S2178 and chromosome 10p between D10S1477 and D10S601. Heterogeneity testing strongly supports linkage for glaucoma to at least one of these regions and suggests possible linkages to both. Although TIGR/myocilin and optineurin mutations have been shown to be causally linked to POAG in other populations, findings from this study do not support either of these as causative genes in an Afro-Caribbean population known to have relatively high rates of POAG.

Introduction

Glaucoma is a combination of diseases causing optic neuropathy and is characterized by optic disc cupping and loss of visual fields. There are several types of glaucoma, including juvenile and adult-onset types, such as primary open-angle, narrow-angle, and secondary glaucoma, each thought to have different pathophysiological mechanisms. Although the inheritance patterns and genetic contributions for congenital glaucoma have been well-characterized, the underlying cause of primary open-angle glaucoma (POAG) remains unknown.

POAG is the most common type of glaucoma and is one of the leading causes of blindness worldwide (Quigley 1996); its prevalence rises steeply with age, and the disease mainly affects older adults. POAG is more prevalent in black than white populations, with reported frequencies of 4% in Afro-Americans over the age of 40 years, as compared with approximately 1% in white populations (Leske 1983; Tielsch et al. 1991; Leske et al. 1994). The disease is particularly frequent in Afro-Caribbeans, with prevalences of 7% in Barbados (Leske et al. 1994) and 8.8% in St. Lucia (Mason et al. 1989). In addition to racial differences, a positive family history of POAG is a major risk factor for the disease (Shin et al. 1977; Leske 1983; Leske et al. 1995; Tielsch et al. 1994), consistent with genetic factors contributing to POAG. Evidence for POAG genetic susceptibility in the Barbados black population comes from a nested case-control study that found a positive association between POAG and the Duffy Fy^{a+} allele (Leske et al. 1996).

Several genetic loci contributing to POAG susceptibility have been identified. Regions on chromosomes 1q23 (GLC1A; Sheffield et al. 1993), 2cen-q13 (GLC1B; Stoilova et al. 1996), 3q21-q24 (GLC1C; Wirtz et al. 1997), 8q23 (GLC1D; Trifan et al. 1998), 10p15-p14 (GLC1E; Sarfarazi et al. 1998), and 7q35-q36 (GLC1F; Wirtz et al. 1999) have been linked to hereditary glaucoma, in most cases by using a small number of large families exhibiting autosomal dominant inheritance. Wiggs et al. (2000) performed a genome-wide scan for adult-onset POAG based on a sibpair multipoint analysis and reported additional linkage to loci in regions on chromosomes 2, 14, 17, and 19; the region on chromosome 2 does not overlap with GLC1B. To date, the genes that underlie two of the six named POAG loci have been identified: TIGR/myocilin is the gene for GLC1A (Stone et al. 1997) and optineurin (OPTN) is the gene for GLC1E implicated in low tension glaucoma (Rezaie et al. 2002), whereas genes underlying the other reported loci identified by linkage analysis have not yet been identified.

The Barbados Family Study of Open-Angle Glaucoma (BFSG) was designed to identify specific loci associated with adult-onset glaucoma in a large number of families of African origin. A previously conducted segregation analysis in this population indicated that POAG is likely to follow a codominant mode of inheritance (Nemesure et al. 2001). Using the parameter estimates from this segregation analysis, we performed a genome screen of all informative families and carried out additional genotyping and refined mapping in regions indicating the likelihood of linkage.

Materials and methods

BFSG centers

The BFSG was funded by the National Eye Institute, Bethesda, Maryland and represents a collaboration among four study centers: the Coordinating Center, Stony Brook, New York (study design, data processing and management, analyses); the Data Collection Center, Barbados, West Indies (recruitment and examinations); the Laboratory Center, Bethesda, Maryland (genotyping, analyses); and the Reading Center, Baltimore, Maryland (grading fundus photographs). Informed consent was obtained from all BFSG participants, and the study protocols conformed to the Declaration of Helsinki.

Study population

The BFSG is a follow-up to the Barbados Eye Study (BES), 1988–1992 and the Barbados Incidence Study of Eye Diseases (BISED), 1992–1997, which aimed at evaluating the prevalence, incidence, and risk factors for major causes of visual loss in Barbados, West Indies. Individuals of African descent found to have POAG in the BES/BISED were invited to participate in the BFSG. Additional cases were identified from the glaucoma clinic of the Queen Elizabeth Hospital, Bridgetown, Barbados. All probands were asked to provide demographic and ocular history data, and contact information for their relatives. First-degree relatives of affected probands were invited to participate in the study, as were first-degree relatives of family members found to have glaucoma. Eligible family members were required to be 25 years of age or older and living in Barbados at the time of the study. The recruitment of BFSG families has been detailed elsewhere (Leske et al. 2001a).

All participants received a comprehensive examination including anthropometric and blood pressure measurements, best corrected visual acuity based on the ETDRS chart, Humphrey perimetry with the C64 suprathreshold program, C24–2 and C30–2 full threshold programs, applanation tonometry, pupil dilation, lens gradings with the lens opacities classification system II (Chylack et al. 1989) at the slit lamp, and color stereo fundus photographs of the disc and macula. All participants also received a comprehensive examination by the study's ophthalmologists and an interview including medical, ocular, and family history information. A blood sample of 14 ml was obtained from all available members in each family, including affected and unaffected individuals. Guthrie cards were used on the infrequent occasions when venipuncture could not be performed. DNA was isolated and a sample from at least one affected individual from each family was transformed to create lymphoblastoid lines. Genomic DNA was isolated directly from blood and from transformed lymphocytes as described elsewhere (Smith et al. 1992).

POAG diagnostic criteria

We defined POAG as the presence of visual field and optic disc abnormalities in at least one eye, after the exclusion of other possible causes, as noted by the BFSG ophthalmologists. Table 1 presents the specific criteria used to classify glaucoma status in this study (Leske et al. 2001a) and represents the standard criteria developed and published by the BES (Leske et al. 1994, 2001b). Since some participants were not able to complete all of the protocols (because of advanced visual loss), data completeness was taken into account in the classification scheme. Double plus (++) signs denote the most complete data and single plus (+) signs indicate less complete data, with both being sufficient for the classification of glaucoma. Those with POAG had a minimum of at least one plus (+) sign in *each* of the three categories (visual field, optic disc, and ophthalmologic evaluation). Those who met some but not all of the POAG criteria were considered glaucoma suspects (GS). We further subcategorized the glaucoma suspects into two groups. Participants with a *cumulative total* of three or more plus (+) signs in two of the three categories were thought to have a more advanced condition and were classified as GS-1. Those with fewer than three plus (+) signs and the presence of some glaucomatous pathology were considered to be GS-2. Individuals not meeting the criteria for glaucoma or suspect glaucoma were classified as unaffected for the purposes of the linkage analysis. Whereas some of these individuals might have had elevated intraocular pressure, they did not meet the strict study criteria for affectedness used in both the linkage analysis and the preceding segregation study (Nemesure et al. 2001). All individuals genotyped and used in the linkage analysis,

Table 1 Diagnostic criteria for primary open-angle glaucoma (POAG); ++ most complete classification data, + less complete data, but sufficient for classification

Classification	Criteria
Visual field	
++	At least two abnormal visual field tests by Humphrey automated perimetry, as defined by computer-based objective criteria, i.e., positive results of hemimeridional analyses of threshold tests (C24–2 or C30–2 full-threshold program) and/or the presence of one or more absolute defects in the central 30 degrees (as tested with the C64 suprathreshold program; 3-zone strategy), with ophthalmologic interpretation as definite or suspect glaucomatous field loss
+	Less than two abnormal visual field tests or an inability to perform reliable automated perimetry (because of severe visual impairment or infirmity), with ophthalmologic interpretation as definite glaucomatous field loss
Optic disc	
++	At least two signs of optic disc damage present in fundus photographs and/or the ophthalmologic evaluation, including either a horizontal or vertical cup-disc ratio ≥0.7, narrowest remaining neuroretinal rim ≤0.1 disc diameters, notching, asymmetry in cup-disc ratios between eyes >0.2, or disc hemorrhages
+	Less than two signs of optic disc damage as described above (or unavailable photographs), with an ophthalmologic assessment or clinical record documenting definite glaucomatous optic nerve damage
Ophthalmologic examination	
++	Clinical diagnosis of definite POAG after examination by the study ophthalmologist to exclude other possible causes for disc and field changes
+	Previous POAG history and treatment and/or visual field and disc damage, although a definite POAG diagnosis was not made at the time of the BFSG visit (e.g., because of inconclusive or incomplete data); the study ophthalmologist confirmed the diagnosis through record review or re-examination

both affected and unaffected, underwent the same detailed ophthalmological examination described above.

Among relatives with suspect glaucoma in this study, 78% had optic disc pathology consistent with glaucoma (but did not meet the conservative visual field criteria), whereas the remaining 22% exhibited convincing visual field damage attributed to glaucoma by the ophthalmologists (but did not meet the study criteria for optic disc damage). These findings, along with the finding that 26% of persons in the BES with a baseline diagnosis of suspect glaucoma were found to have POAG 4 years later (Leske et al. 2001b), suggest that persons classified as suspects in this study are likely to have "latent" POAG, which will fully meet our criteria for diagnosis with further follow-up. Because of this conservative definition of glaucoma, anyone with a diagnosis of POAG, GS-1, or GS-2 was therefore classified as "affected".

Our classification of "ocular hypertension" solely included persons with an intraocular pressure (IOP) over 21 mm Hg but without any signs of glaucoma-related optic disc and visual field damage. Incidence data from BISED indicated that among 365 ocular hypertensives (IOP>21 mm Hg and no other glaucomatous pathology) at baseline, only 4.9% (n=18) progressed to POAG after 4 years (Leske et al. 2001b), an estimate in line with the 1%/year overall incidence among ocular hypertensives reported in the literature (Leske 1983; American Academy of Ophthalmology 2002). Therefore, although elevated IOP is a major risk factor for POAG, most persons with high IOP will not develop POAG. Thus, ocular hypertensives were considered "unaffected" in these analyses.

Family structure

A summary of the pedigree structures of families used in the linkage analysis is shown in Table 2. Family sizes ranged from 3 to 26 individuals with an average size of nine individuals per family, of which an average of 2.4 were affected. Of the 146 total families analyzed, 32 were nuclear, 26 extended vertically over more than two generations, 33 involved multiple matings in one generation, and 55 both extended over multiple generations and involved multiple matings in one generation. Seven families contained matings in which both parents were affected. Whereas all 1327 individuals (350 of whom were affected) in the pedigrees contributed to the analysis, not all were genotyped. In all, 662 individuals (256 of whom were affected) were genotyped and analyzed throughout the study. This corresponded to 4.5 individuals per family, with an average of 1.7 affected individuals in each family. In summary, 52 families had no affected individuals genotyped, eight families had a single affected individual genotyped, 42 families had two affected individuals genotyped, 26 families had three affected individuals genotyped, seven families had four affected individuals genotyped, nine families had five affected individuals genotyped, and single families had six and seven individuals genotyped. Only individuals meeting the inclusion criteria were analyzed in the study. The average age of individuals registered in the linkage study was 61 years with a standard deviation of 16 years.

Table 2 Distribution of BFSG families by family size and number of affecteds

Family size	Number of affecteds					
	1	2	3	4	5+	Total
3–5	14	13	3	0	0	30
6–9	24	18	14	3	2	61
10+	5	13	17	10	10	55
Total	43	44	34	13	12	146

Genotyping

Genotyping was carried out as described by Jiao et al. (2000). The ABI PRISM Linkage Mapping Set MD-10 (ABI, Foster City, Calif.) comprising 382 microsatellite markers with an average spacing of 10 cM was used. The products were subsequently separated on a 5% denaturing polyacrylamide gel in an Applied Biosystems 377 DNA sequencer. Polymerase chain reactions (PCRs) were prepared following the manufacturer's protocols. Multiplex PCR amplifications were performed in an ABI PRISM 8700 thermocycler workstation. The GENESCAN and GENOTYPER software packages (Applied Biosystems/Perkin-Elmer) were used to analyze alleles. Two technicians, masked to the identity of participants, independently interpreted all gels with conflicts being resolved by a third independent reader. Conflicting results that could not be resolved were discarded or, in an area of interest, repeated. Family relationships were confirmed by observation of Mendelian inheritance of alleles of microsatellite markers from all panels of the ABI Linkage Mapping Set MD-10, which also provided significant error-checking for the genotyping. Spot checks of genotyping were also performed for a limited number of markers including those on chromosome 2 by comparing intermarker distances calculated from the linkage data to known map distances from the CHLC map. In regions with MD-10 markers having LOD scores >1.0, additional surrounding microsatellite markers from the Genethon map (ftp://ftp.genethon.fr/pub/Gmap/Nature-1995/data/; Dib et al. 1996) were analyzed in a similar fashion.

Linkage analysis

For the linkage analysis, individuals evaluated at the clinic and considered unaffected were coded as "1" (unaffected), whereas those classified as OAG, GS-1, or GS-2 were coded as "2" (affected). Linkage calculations were performed with FASTLINK version 4.1P (Cottingham et al. 1993; Schäffer et al. 1994), a faster version of LINKAGE 5.1 (Lathrop and Lalouel 1984). The genotyping data were analyzed under autosomal dominant models (with and without age adjustment) and under a codominant model. Individuals in various diagnostic categories were assigned to penetrance classes as shown in Table 3, and these penetrance values were derived from the segregation analysis described previously (Nemesure et al. 2001) and adjusted for age. The penetrance values used in the analysis allow for substantial diagnostic uncertainty (Terwilliger and Ott 1994), and hence the analysis is not vitiated by the fact that a few individuals classified as unaffected will go on to develop glaucoma. As mentioned above, the unaffected individuals were coded as having affection status 1, whereas the GS-1, GS-2, and OAG individuals were coded as having affection status 2, so that the same penetrance values have distinct and complementary roles in the likelihood calculation. Since the phenocopy rates are at least 0.14, which is much higher than any reasonable mutation rates, and none of the penetrances are 1.0, we ignored the possibility of mutations in the inheritance model. Marker allele frequencies were estimated with DOWNFREQ of the ANALYZE package (Terwilliger 1995); DOWNFREQ does not take disease status into account. In an initial check of the data, we performed a linkage analysis with an autosomal dominant mode of inheritance and one penetrance class with values 0.05, 0.75, 0.75, respectively. The results of this analysis were not qualitatively different from the analysis performed with the dominant model parameters selected via the segregation analysis, and hence findings from the simpler model are omitted. Two-point analyses were carried out with the MLINK and ILINK programs of FASTLINK. Regions containing markers that yielded LOD scores >1.0 were further analyzed by multipoint analyses by using the LINKMAP program of FASTLINK with intermarker recombination fractions taken from the Marshfield map (Broman et al. 1998). Model-free linkage analysis was carried out with SimIBD version 2.1 (Davis et al. 1996) and these analyses were restricted to families with ≥2 affecteds, as the method is undefined for families with <2 affected. *P*-values <0.05 from SimIBD were considered significant. Locus-heterogeneity tests were carried out with the HOMOG program (Ott 1999). Replicates of unlinked families used to test the significance of LOD scores by simulation were generated by using FASTSLINK (Ott 1989; Weeks et al. 1990; Cottingham et al. 1993), and LOD scores in the replicates were calculated by using FASTLINK (Cottingham et al. 1993). Allelic association was assessed with the TRANSMIT program (Clayton 1999).

PCR amplification was carried out in a Perkin-Elmer 9700 thermocycler with 100 ng genomic DNA amplified in GeneAmp PCR reaction buffer (Applied Biosystems, Foster City, Calif.) with 1 U *Taq*-Gold DNA polymerase (Applied Biosystems) and 10 pmole of the above primers in a reaction volume of 20 µl. Samples were subjected to a PCR amplification protocol beginning with a denaturation step at 94°C for 8 min, followed by 35 cycles, each consisting of a denaturation step at 94°C for 30 s, an annealing step at 55°C for 30 s, and extension step at 72°C for 40 s, followed by a final extension at 72°C for 3 min.

Table 3 Penetrance estimates/liability classes (α allele frequency, *POAG* primary open-angle glaucoma, *GS-1* glaucoma suspect [advanced], *GS-2* glaucoma suspect [early])

Liability class	Gender	Penetrance		
Dominant model (α=0.029)				
Unaffected	Male	0.20	0.99	0.99
	Female	0.14	0.83	0.83
POAG	Male	0.20	0.99	0.99
	Female	0.14	0.83	0.83
GS-1	Male	0.20	0.74	0.74
	Female	0.14	0.62	0.62
GS-2	Male	0.20	0.49	0.49
	Female	0.14	0.41	0.41
Codominant model (α=0.181)				
Unaffected	Male	0.01	0.54	0.99
	Female	0.01	0.27	0.98
POAG	Male	0.01	0.54	0.99
	Female	0.01	0.27	0.98
GS-1	Male	0.01	0.40	0.74
	Female	0.01	0.20	0.74
GS-2	Male	0.01	0.27	0.49
	Female	0.01	0.14	0.49

DNA sequencing

PCR products were analyzed on 1% agarose gels and purified by using a Quick Step 2 96-well PCR purification kit (Edge Biosystems, Gaithersburg, Md.) and concentrated by ethanol precipitation. Sequencing in the forward and reverse directions with the same exon-specific primers as were used for PCR amplification was performed by using an Amplitaq FS cycle sequencing kit (ABI) with dye-labeled terminators according to the manufacturer's instructions. Sequencing reactions were purified by using the Performa DTR 96-well short plate kit (Edge Biosystems), and sequences were analyzed on an ABI 3100 DNA analysis system. Myocilin was sequenced as previously described (Stone et al. 1997).

Results

Family recruitment

The BFSG initially identified 230 probands and 1377 relatives who have been described in detail elsewhere (Nemesure et al. 2001). In brief, 94% of the probands

Table 4 Two–point LOD scores >1.0

Marker	Model	LOD score	θ
D1S2785	Dominant with age adjustment	1.13	0.00
D2S364	Codominant	1.70	0.15
D2S117	Codominant	1.78	0.15
D2S325	Codominant	1.74	0.15
D9S290	Dominant with age adjustment	1.02	0.00
D10S192	Dominant without age adjustment	1.16	0.00
D10S548	Codominant	2.14	0.00
D11S937	Dominant without age adjustment	1.15	0.03
	Dominant with age adjustment	1.02	0.03
D14S283	Dominant with age adjustment	1.18	0.00
D14S288	Dominant without age adjustment	1.11	0.07

completed at least one visual field test, 99% completed ophthalmologic or photographic disc gradings, and 99% completed ophthalmologic examinations. Of 1377 eligible relatives, 1071 (78%) participated in the study, with similar numbers completing the clinical assessment. When corrected for age, BFSG relatives were twice as likely as BES participants overall to have POAG, and four times as likely to meet the criteria for suspected POAG. The probands had a mean age of 68 years, whereas relatives had a younger mean age of 47 years; 52% of the probands were male, whereas 41% of the relatives were male.

Because the families in the BFSG were selected inclusively to be appropriate for genetic epidemiological analysis, they included a number of small nuclear families with single affected individuals. Before genotyping was initiated, families containing two or more affected individuals or a single individual with three or more unaffected siblings were selected for analysis. In addition, some pedigrees required revision as non-Mendelian inheritance patterns for marker alleles were uncovered (see above). Finally, extension of the initial pedigrees allowed four nuclear families to be joined into two extended families. After these revisions, the total number of individuals included in the study was 1327, composing 146 families, with 350 POAG, GS-1, or GS-2 individuals. Among the 146 families, 103 multiplex families had two or more affected members (n=307 affected). The overall total of 1327 included some individuals who were not examined but were used only in the linkage analysis to connect pedigrees or to provide every nonfounder with two parents. Of the 1327 individuals, 662 were genotyped, and 665 were not genotyped. The main linkage analysis was based on the 146 families.

Myocilin and the initial genome screen

Since TIGR/myocilin has been shown to cause some cases of autosomal dominant open angle glaucoma (Stone et al. 1997), and we knew this at the start of the project, preliminary analyses of this gene were carried out in a subset of our patient population. Sharing of parental alleles at six markers closely linked to GLC1A by 20 pairs of affected siblings (a total of 39 individuals in seven families) was essentially what was expected by random chance (data not shown). The myocilin gene was sequenced in 19 individuals from Barbados. Six affected and three unaffected individuals showed no sequence changes. Three affected and four unaffected individuals showed polymorphisms that did not change the amino acid sequence. One affected and two control individuals showed a base change resulting in a conservative E323 K amino acid sequence change. Overall, whereas these studies did not exclude a role for TIGR/myocilin in glaucoma in Barbados, they did not support a major role for myocilin as a cause of progressive POAG in the Barbados population. In addition, samples from 100 affected and 100 unaffected unrelated controls were analyzed for the markers included in ABI panels 1 and 2, which together cover chromosome 1, and glaucoma did not show allelic association with markers near the myocilin region. Finally, the genome scan was initiated on chromosome 1 and markers flanking myocilin did not show evidence of linkage to glaucoma (data not shown).

An initial genome screen with the ABI MD-10 marker set, giving a 10 cM average grid, was analyzed using the three models described above. Chromosomal regions in which analysis with any of the models yielded a LOD score >1.0 (a threshold also used by Wiggs et al. 2000) are shown in Table 4. Although a number of markers showed some initial promise, markers in three regions were judged promising enough to carry out fine mapping.

D1S2785 gave a LOD score of 1.13 at θ=0.00 under the autosomal dominant inheritance model with an age-dependent penetrance derived from the segregation analysis. However, when analyzed by multipoint analysis with the flanking markers, the LOD score dropped well below 1, and this region was not pursued.

The three positive markers on chromosome 2 are adjacent in the marker set: D2S364 — D2S117 — D2S325. Multipoint analysis with all three markers gave a maximum LOD score of 1.96 under the codominant model with the map Glc — D2S364 — D2S117 — D2S325. In addition, analysis by using SimIBD gave empirical P-values of 0.11, 0.95, and 0.03 for D2S364, D2S117, and D2S325, respectively. This interval on chromosome 2 is not near the GLC1B locus or the possible glaucoma locus on chromosome 2 suggested by Wiggs et al. (2000).

D9S290 gave a two-point LOD score of 1.02 at θ=0.00 and multipoint scores close to the threshold of 1.0. Although D9S290 is about 5 Mb from the LMX1B gene

Table 5 Fine mapping of chromosome 2 (θ recombination fraction, Z_{max} maximum LOD score)

Distance from 2ptel	Marker	θ							Z_{max}	Best θ	Z_{max} under heterogeneity	SimIBD *P*-value
		0	0.01	0.03	0.05	0.07	0.1	0.15				
182.24	D2S2314	–0.40	–0.17	0.22	0.53	0.77	1.01	1.20	1.22	0.17		
184.04	D2S324	–0.31	–0.06	0.34	0.65	0.87	1.09	1.23	1.23	0.16		
186.21	D2S364[a]	0.65	0.84	1.15	1.38	1.54	1.67	1.69	1.71	0.13	2.04	0.105
188.11	D2S152	1.46	1.54	1.66	1.74	1.79	1.80	1.70	1.80	0.09	1.85	0.019
190.00	D2S118	–1.05	–0.83	–0.46	–0.16	0.08	0.34	0.59	0.66	0.20		
191.08	D2S161	–0.85	–0.65	–0.30	–0.01	0.23	0.51	0.79	0.90	0.21		
193.26	D2S2167	0.40	0.54	0.77	0.93	1.04	1.13	1.13	1.15	0.122		
194.45	D2S117[a]	0.72	0.95	1.31	1.58	1.77	1.93	1.95	1.97	0.13	2.09	0.954
196.85	D2S311	–0.19	0.02	0.35	0.61	0.80	0.99	1.11	1.11	0.16		
199.18	D2S2189	2.32	2.45	2.63	2.73	2.77	2.73	2.49	2.77	0.08	2.91	0.143
200.43	D2S2237	–3.19	–2.81	–2.18	–1.66	–1.24	–0.75	–0.19	0.25	0.28		
202.92	D2S369	–1.52	–1.28	–0.86	–0.52	–0.24	0.09	0.42	0.57	0.23		
204.53	D2S325[a]	1.39	1.54	1.78	1.95	2.06	2.13	2.05	2.14	0.11	2.13	0.026
205.59	D2S2178	–2.10	–1.80	–1.30	–0.89	–0.57	–0.20	0.18	0.39	0.24		
206.74	D2S371	–0.46	–0.15	0.34	0.71	0.98	1.26	1.44	1.45	0.16		

[a]Used in genome scan

(Dreyer et al. 1998; Vollrath et al. 1998), we are not aware of any LMX1B mutations that are implicated in glaucoma without co-occurrence of nail-patella syndrome (Lichter et al. 1997). As such, the neutral results for D9S290 were not further pursued.

On chromosome 10, D10S548 gave a LOD score of 2.14 at θ=0.00 under the codominant inheritance model. This was of particular interest because D10S197, located 6.8 cM telomeric from D10S548, gave a LOD score of 0.72 at a θ=0.15. However, multipoint analysis with these two markers gave a LOD score of only 1.39. This region was also of interest because it was near, though did not overlap, the GLC1E locus (Sarfarazi et al. 1998).

Two markers from chromosome 14 gave two-point LOD scores >1.0. D14S283 gave a LOD score of 1.18 at θ=0.00 under the dominant model with age adjustment. D14S288 gave a LOD score of 1.11 at θ=0.07 (under the dominant model without age adjustment) by using an affected allele frequency of 0.09, which maximizes the multipoint LOD score with D14S70 and D14S288 to 1.91. SimIBD did not give any statistically significant results for these markers.

Although markers in one region of chromosome 18 gave two-point LOD scores close to 1.0, and the multipoint analyses of D18S68 and D18S61 (which are adjacent markers lying 9.8 cM apart) gave LOD scores of 1.12 and 1.26 under the codominant model, this region was not pursued further because of negative scores at flanking markers and limited resources.

Fine mapping of chromosomes 2, 10 and 14

Additional markers were genotyped in the regions of chromosomes 2, 10, and 14 described above. In addition, any genotypes that had been discarded as unreadable in the initial genomic screen were repeated, so that data were essentially complete for each sampled individual and each marker.

The region of chromosome 14 spanning D14S283 and D14S288 was further examined by using nine additional markers: D14S972, D14S1032, D14S80, D14S1040, D14S1060, D14S1014, D14S1039, D14S976, and D14S1031. This region was also of interest because it overlaps one of the highest scoring regions in the study by Wiggs et al. (2000). Therefore, we decided to vary the disease allele frequency to assess whether the modestly positive scores were partly attributable to a mis-specification of the model. This gives a more full assessment of whether our results replicate the prior linkage claim. We make no claim of glaucoma linkage to chromosome 14 based on our data alone.

The results indicate that the maximum two-point LOD scores in this region of chromosome 14 were obtained with D14S1039 analyzed under the dominant model with age effects and an allele frequency of 0.125 (Z_{max}=1.56 at θ=0.07) and with D14S1031 under the codominant model with a disease allele frequency of 0.005 (Z_{max}=2.02 at θ=0.186). The LOD score under locus heterogeneity did not increase. Thus, whereas the chromosome 14 region cannot be excluded, there is no definitive evidence of linkage. Some of these results could be construed as giving weak support to the results of Wiggs et al. (2000) suggesting that there is a glaucoma locus near D14S283.

Microsatellite markers used for fine mapping of chromosome 2, their chromosomal position, and the two-point LOD scores obtained with the codominant model are shown in Table 5. Only the codominant model gave good scores for this region in the genome scan analysis, so only the codominant model was used in analyzing the fine mapping data. We did not vary the model parameters, so as not to do multiple testing, other than for multiple markers. The markers are an average of 2–3 cM apart. All of the markers in this region gave positive LOD scores, most

Table 6 Fine mapping of chromosome 10 (θ recombination fraction, Z_{max} maximum LOD score)

Distance from 10ptel	Marker	θ							Z_{max}	Best θ	Z_{max} under heterogeneity	SimIBD *P*-value
		0	0.01	0.03	0.05	0.07	0.1	0.15				
40.36	D10S1653[a]	–4.11	–3.74	–3.11	–2.59	–2.15	–1.61	–0.98	0.00	0.50		
42.50	D10S1477	–2.95	–2.57	–1.94	–1.44	–1.04	–0.58	–0.11	0.20	0.26		
45.70	D10S548[a]	2.20	2.18	2.12	2.05	1.96	1.80	1.50	2.20	0.00	2.20	0.36
45.70	D10S1714	–1.38	–1.12	–0.68	–0.32	–0.03	0.28	0.57	0.65	0.20		
48.36	D10S211	3.12	3.18	3.24	3.25	3.20	3.07	2.70	3.26	0.04	3.30	0.13
49.43	D10S1789	–1.40	–1.08	–0.58	–0.20	0.09	0.40	0.66	0.71	0.19		
52.10	D10S197[a]	–1.02	–0.76	–0.34	–0.02	0.23	0.50	0.72	0.76	0.19		
54.23	D10S600	1.04	1.21	1.48	1.66	1.77	1.85	1.78	1.85	0.11	1.85	0.22
57.42	D10S601	–2.35	–2.00	–1.42	–0.95	–0.57	–0.14	0.31	0.55	0.24		

[a]Used in genome scan

of them being over 1. The markers in this interval giving the highest LOD scores are D2S2189 (Z_{max}=2.77 at θ=0.08), D2S325 (Z_{max}=2.14 at θ=0.11), and D2S117 (Z_{max}=1.97 at θ=0.13). LOD scores computed under the assumption of heterogeneity were only slightly increased relative to those calculated assuming homogeneity.

Three-point LOD scores calculated by using markers D2S152 and D2S2189 gave a maximum LOD score of 3.31 (3.48 under heterogeneity), with D2S117 and D2S2189 gave a LOD score of 3.11 (3.51 under heterogeneity), and with markers D2S2189 and D2S325 gave 3.37 (3.40 under heterogeneity). Other three-point LOD scores were slightly lower: D2S152 and D2S117 gave 2.36, D2S152 and D2S325 gave 3.02, and D2S117 and D2S325 gave 2.70, all with small increases of 0.05–0.4 under heterogeneity. Four-point analysis with markers D2S364-D2S117-D2S325 gave a maximum LOD score of 2.64, whereas four-point analysis with markers D2S364-D2S117-D2S2189 gave 3.27.

We used FASTSLINK to generate 3000 unlinked replicates with allele frequencies of D2S2189 and then computed the best LOD score for each replicate. No replicates exceeded the true LOD score of 2.77, but one replicate had a LOD score of 2.76. Treating this conservatively as if one replicate tied or exceeded the true LOD score gives an empirical P<0.0017 for the two-point results obtained with this marker. Analysis by using SimIBD gave P-values of 0.019 for the results of D2S152, and 0.026 for the results of D2S325, but only 0.143 for D2S2189, and 0.105 for D2S364, whereas D2S117 gave 0.954. Analysis of transmission disequilibrium by using the TRANSMIT program (Clayton 1999) gave minimally significant results for undertransmission of one allele at D2S2189 (χ^2= 7.8161, 1 df) and one allele at D2S311 (χ^2=8.6858, 1 df), but these were not significant after correction for the number of alleles and markers tested.

For each of the markers reported in Tables 4 and 5, we tested for an unexpected number of homozygotes by sampling the first genotyped individual in each pedigree and performing a two-sided χ^2 test. None of the 15 markers on chromosome 2 had a nominal p-value <0.1. Among the chromosome 10 markers, D10S1789 had a nominal P <0.004, D10S197 had a nominal P <0.08, and the other seven markers each had a nominal P >0.2. The low P-value for D10S1789 is attributable to an excess of homozygotes that might indicate some genotyping problems with that marker, but the LOD scores that we obtained for D10S1789 are low and the nominal P <0.004 is not significant when corrected for multiple testing.

The microsatellite markers used to examine further the linked region on chromosome 10 are shown in Table 6. Similar to the 2q region, the average distance between markers was 2–3 cM, and we used only the codominant model for the chromosome 10 region, without varying the model parameters. Positive LOD scores were seen when the data obtained with D10S548 (Z_{max}=2.2 at θ=0.00), D10S211 (Z_{max}=3.26 at θ=0.04), and D10S600 (Z_{max}=1.85 at θ=0.11) were analyzed by using the codominant model. These LOD scores did not improve significantly when analyzed under heterogeneity by using HOMOG. Three-point LOD scores computed by using the markers D10S548 and D10S211 were 4.09 (4.16 under heterogeneity), with D10S548 and D10S600 were 1.89 (1.99 under heterogeneity), and with D10S211 and D10S600 were 2.34 (2.35 under heterogeneity). However, the four-point LOD score with all three markers decreased to 2.51. A simulation study with FASTSLINK by using the allele frequencies of D10S211 yielded 0 replicates out of 3000 with a LOD score above 3.26 for an empirical P <0.001. SimIBD analysis gave no significant results, and therefore our findings on chromosome 2 may be viewed as more likely being a true positive than our result on chromosome 10. In addition, there was no allelic association for alleles at the chromosome 10 markers, as assessed by using TRANSMIT.

For completeness, we performed additional linkage analyses for chromosomes 2 and 10, on only the 103 multiplex families. The results indicated that the highest LOD scores were slightly lower than those obtained with all 146 families but were still supportive and high enough to be of interest (data not shown).

Testing for heterogeneity

The relatively small increases in LOD scores obtained when the genotypes of markers in the linked regions of chromosomes 2 and 10 were further analyzed by the admixture test with HOMOG provided little evidence for locus heterogeneity. However, a heterogeneity test by using the 2q and 10p linkage regions with HOMOG3R showed evidence for heterogeneity and even stronger evidence for linkage of one or both loci. HOMOG3R tests the hypothesis that each family is linked to one locus of two loci versus the null hypothesis of no linkage to either locus. HOMOG3R was run with D2S2189 representing the chromosome 2 locus and D10S211 representing the chromosome 10 locus. The best estimate, to within 1%, was that 45% of families were linked to chromosome 2 and 55% of families to chromosome 10. The LOD score for the hypothesis that some families are linked to chromosome 2 and other families to chromosome 10 versus the null hypothesis of no linkage is 5.18. The LOD score for the hypothesis that some families are linked to the chromosome 2 locus and other families to the chromosome 10 locus versus the alternative hypothesis that all families are linked to one locus or that all families are linked to the other locus is 1.92. The LOD score for the hypothesis that some families are linked to the chromosome 2 locus and other families to the chromosome 10 locus versus the alternative hypothesis that some families are linked to the chromosome 2 locus and others are unlinked, or that some families are linked to the chromosome 10 locus and others are unlinked is 1.88. The HOMOG3R results strongly support linkage of loci increasing the risk for glaucoma to at least one of the regions on chromosomes 2 or 10 and suggest they may be linked to both.

Mutational screening of OPTN

The centromeric marker flanking the linked region on chromosome 10, D10S1653, lies within about 2 Mb of OPTN, mutations of which have recently been shown to be associated with normal tension glaucoma (Rezaie et al. 2002). The OPTN gene was sequenced in one affected individual from each of the 48 families most likely to be linked to the chromosome 10 region on the basis of the HOMOG3R analysis with D2S2189 and D10S211 ($0.58 > P > 0.88$), and exons in which sequence changes were detected were sequenced in 48 normal controls from the Barbados population (Table 7). Four nucleotide sequence changes were seen, only one of which resulted in an amino acid change. This was a conservative E323K change seen in four affected individuals and four controls. In addition, there were two base changes noted incidentally in introns: a T to C transition 5 bp 5' of exon 7 in a single affected individual and a C to T transition 30 bp 3' of exon 12 in a single unaffected individual.

Table 7 Mutational screening of OPTN (*NCBI* National Center for Biotechnology Information)

Nucleotide change (NCBI)	Protein change (NCBI)	Affected	Unaffected
G256A	T35T	5	6
C556A	A135A	1	0
G1118A	E323K	4	4
C1117T	S322S	0	4

Discussion

Findings from the BFSG indicate that POAG in the black population of Barbados may be linked to regions on chromosome arms 2q and 10p, with multipoint LOD scores >3.0 in both cases. These results are based on analyses with the codominant model and associated parameter estimates derived from a previous segregation analysis of these families. Model-free analyses with SimIBD gave significant *P*-values for some markers in the 2q region but not the 10p region. In both cases, simulation studies gave empirical *P*-values <0.002 for the highest two-point LOD scores with the best markers.

Transmission disequilibrium testing shows some evidence of association in the middle of the 2q region but no evidence of association in the 10p region. The 10p region is near optineurin but does not contain optineurin. Both linkage analysis and mutation testing provide strong evidence against optineurin being the primary cause of the linkage result. Additionally, the BFSG family data do not provide evidence to support TIGR/myocilin as a causal factor for POAG. There was an absence of allelic association with markers in the myocilin region on chromosome 1, and markers flanking this region were not found to be linked with POAG.

In conclusion, the BFSG results provide evidence for linkage of POAG to chromosome 2 or chromosome 10, and possibly both. In early 2002, we looked at the public draft human genome assembly for candidate genes in the two linkage regions. In the 2q region, we found CYP-M, a relative of CYP1B1, and a retina-specific protein MPP4 (Stöhr and Weber 2001), which are both close to the markers that showed minimal evidence of association with glaucoma. In the 10p region, we found MYO3A and PIP5K2A, but both regions contain many as yet uncharacterized genes. The findings presented in this report require further replication or substantiation by the identification of causative genes.

Acknowledgements We wish to thank the BFSG participants, the Queen Elizabeth Hospital, and the Ministry of Health, Barbados, West Indies for their role in the study. In addition, we are grateful to the Computational Methods and Algorithms Group in the Division of Computational Bioscience at the Center for Information Technology of the National Institutes of Health (NIH) for providing high-performance computational resources for this study. We should also like to acknowledge Dr. Edwin Stone at the University of Iowa for his genotyping assistance in a sample of participants, and the expert technical assistance of Ms. Chalani De Silva at the NIH and her student assistants, Ms. Chamari De Silva and Catalina Teba.

The BFSG Study Group
M. Christina Leske, MD, MPH (Principal Investigator)
Coordinating Center—University at Stony Brook, Stony Brook, NY
M.C. Leske, MD, MPH; Barbara Nemesure, PhD; Qimei He, PhD; Suh-Yuh Wu, MS; Nancy Mendell, PhD; Lixin Jiang, MS; Kasthuri Sarma; Koumudi Manthani.
Data Collection Center—Ministry of Health, Bridgetown, Barbados, West Indies
Anselm Hennis, MRCP (UK), PhD; Ann Bannister, MB, BS, DO, MRCOphth (UK); Muthu Thangaraj, MB, BS, DO; Rajiv Luthra, MD, MPH; Coreen Barrow; Anthanette Holder.
Fundus Photography Center—The Johns Hopkins University, Baltimore, MD
Andrew P. Schachat, MD; Judith A. Alexander; Deborah Phillips; Reva Ward-Strozykowski.
Laboratory Center—The National Eye Institute, Bethesda, MD
James Fielding Hejtmancik, MD, PhD; Joy Redman, MS; Xiadong Jiao, PhD.
Local Advisory Group—Trevor Hassell, MBBS, FRCP, FACC, GCM (Department of Cardiology) and Henry Fraser, FACP, FRCP (UK), PhD, GCM (Chronic Diseases Research Centre) School of Clinical Medicine and Research, University of the West Indies; Clive Gibbons, FRCS (Ed), FRCP, FCOph (UK) (Department of Ophthalmology) Queen Elizabeth Hospital, Barbados, West Indies.

References

American Academy of Ophthalmology (2002) Primary open-angle glaucoma suspect, preferred practice pattern. American Academy of Ophthalmology, San Francisco

Broman KW, Murray JC, Sheffield VC, White RL, Weber JL (1998) Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet 63: 861–869

Chylack LT Jr, Leske MC, McCarthy D, Khu P, Kashiwagi T, Serduto R (1989) Lens opacities classification system II (LOCS II). Arch Ophthalmol 107:991–997

Clayton D (1999) A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. Am J Hum Genet 65:1170–1177

Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53:252–263

Davis S, Schroeder M, Goldin LR, Weeks DE (1996) Nonparametric simulation-based statistics for detecting linkage in general pedigrees. Am J Hum Genet 58:867–880

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Dreyer SD, Zhou G, Baldini A, Winterpacht A, Zabel B, Cole W, Johnson RL, Lee B (1998) Mutations in *LMX1B* cause abnormal skeletal patterning and renal dysplasia in nail patella syndrome. Nat Genet 19:47–50

Jiao X, Munier FL, Iwata F, Hayakawa M, Kanai A, Lee J, Schorderet DF, Chen M-S, Kaiser-Kupfer M, Hejtmancik JF (2000) Genetic linkage of Bietti crystallin corneoretinal dystrophy to chromosome 4q35. Am J Hum Genet 67:1309–1313

Lathrop GM, Lalouel JM (1984) Easy calculations of lod scores and genetic risks on small computers. Am J Hum Genet 36: 460–465

Leske (1983) The epidemiology of open-angle glaucoma: a review. Am J Epidemiol 118:166–191

Leske MC, Connell AMS, Schachat AP, Hyman L, Barbados Eye Study Group (1994) The Barbados Eye Study: prevalence of open-angle glaucoma. Arch Ophthalmol 112:821–829

Leske MC, Connell AMS, Wu S-Y, Hyman LG, Schachat AP, Barbados Eye Study Group (1995) Risk factors for open-angle glaucoma: the Barbados Eye Study. Arch Ophthalmol 113:918–924

Leske MC, Nemesure BB, He Q, Mendell N, Polednak A, Barbados Eye Study Group (1996) Open-angle glaucoma and blood groups: the Barbados Eye Study. Arch Ophthalmol 114:205–210

Leske MC, Nemesure B, He Q, Wu S-Y, Hejtmancik JF, Hennis A, Barbados Family Study Group (2001a) Patterns of open-angle glaucoma in the Barbados Family Study. Ophthalmology 108:1015–1022

Leske MC, Connell AMS, Wu S-Y, Nemesure B, Li X, Schachat A, Hennis A, Barbados Eye Studies Group (2001b) Incidence of open-angle glaucoma: the Barbados Eye Studies. Arch Ophthalmol 119:89–95

Lichter PR, Richards JE, Downs CA Stringham HM, Boehnke M, Farley FA (1997) Cosegregation of open-angle glaucoma and the nail-patella syndrome. Am J Ophthalmol 124:506–515

Mason PR, Kosoko O, Wilson MR, Martone JF, Cowan CL Jr, Gear JG, Ross-Degnan D (1989) National survey of the prevalence and risk factors of glaucoma in St. Lucia, West Indies, part I: prevalence findings. Ophthalmology 96:1363–1368

Nemesure B, He Q, Mendell N, Wu S-Y, Hejtmancik JF, Hennis A, Leske MC, Barbados Family Study Group (2001) Inheritance of open-angle glaucoma in the Barbados Family Study. Am J Med Genet 103:36–43

Ott J (1989) Computer-simulation methods in human linkage analysis. Proc Natl Acad Sci USA 86:4175–4178

Ott J (1999) Analysis of human genetic linkage, 3rd edn. Johns Hopkins University Press, Baltimore

Quigley H (1996) Number of people with glaucoma worldwide. Br J Ophthalmol 80:389–393

Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, Héon E, Krupin T, Ritch R, Kreutzer D, Crick RP, Sarfarazi M (2002) Adult-onset primary open-angle glaucoma caused by mutations in optineurin. Science 295:1077–1079

Sarfarazi M, Child A, Stoilova D, Brice G, Desai T, Trifan OC, Poinoosawmy D, Crick RP (1998) Localization of the fourth locus (GLC1E) for adult-onset primary open-angle glaucoma to the 10p15-p14 region. Am J Hum Genet 62:641–652

Schäffer AA, Gupta SK, Shriram K, Cottingham RW (1994) Avoiding recomputation in genetic linkage analysis. Hum Hered 44: 225–237

Sheffield VC, Stone EM, Alward WL, Drack AV, Johnson AT, Streb LM, Nichols BE (1993) Genetic linkage of familial open angle glaucoma to chromosome 1q21-q31. Nat Genet 4:47–50

Shin DH, Becker B, Kolker AE (1977) Family history in primary open-angle glaucoma. Arch Ophthalmol 95:598–600

Smith RJH, Lee EC, Kimberling WJ, Daiger SP, Pelias MZ, Keats BJB, Jay M, Bird A, Reardon W, Guest M, Ayyagari R, Hejtmancik JF (1992) Localization of two genes for Usher syndrome type I to chromosome 11. Genomics 14:995–1002

Stöhr H, Weber BHF (2001) Cloning and characterization of the human retina-specific gene MPP4, a novel member of the p55 subfamily of MAGUK proteins. Genomics 74:377–384

Stoilova D, Child A, Trifan OC, Crick RP, Coakes RL, Sarfarazi M (1996) Localization of a locus (GLC1B) for adult-onset primary open-angle glaucoma to the 2cen-q13 region. Genomics 36:142–150

Stone EM, Fingert JH, Alward WLM, Nguyen TD, Polansky JR, Sunden SLF, Nishimura D, Clark AF, Nystuen A, Nichols BE, Mackey DA, Ritch R, Kalenak JW, Craven ER, Sheffield VC (1997) Identification of a gene that causes primary open-angle glaucoma. Science 275:668–670

Terwilliger JD (1995) A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more marker loci. Am J Hum Genet 56:777–787

Terwilliger JD, Ott J (1994) Handbook of human genetic linkage. Johns Hopkins University Press, Baltimore

Tielsch JM, Sommer A, Katz J, Royall RM, Quigley HA, Javitt JC (1991) Racial variations in the prevalence of primary open-angle glaucoma — the Baltimore Eye Survey. JAMA 266:369–374

Tielsch JM, Katz J, Sommer A, Quigley HA, Javitt JC (1994) Family history and risk of primary open-angle glaucoma. The Baltimore Eye Survey. Arch Ophthalmol 112:69–73

Trifan OC, Traboulsi EI, Stoilova D, Alozie I, Nguyen R, Raja S, Sarfarazi M (1998) A third locus (GLC1D) for adult-onset primary open-angle glaucoma maps to the 8q23 region. Am J Ophthalmol 126:17–28

Vollrath D, Jaramillo-Babb VL, Clough MV, McIntosh I, Scott KM, Lichter PR, Richards JE (1998) Loss-of-function mutations in the LIM-homeodomain gene, *LMX1B*, in nail-patella syndrome. Hum Mol Genet 7:1091–1098

Weeks DE, Ott J, Lathrop GM (1990) SLINK: a general simulation program for linkage analysis. Am J Hum Genet 47:A204

Wiggs JL, Allingham RR, Hossain A, Kern J, Auguste J, DelBono EA, Broomer B, Lennon Graham F, Hauser M, Pericak-Vance M, Haines JL (2000) Genome-wide scan for adult onset primary open-angle glaucoma. Hum Mol Genet 9:1109–1117

Wirtz MK, Samples JR, Kramer PL, Rust K, Topinka JR, Yount J, Koler RD, Acott TS (1997) Mapping a gene for adult-onset primary open-angle glaucoma to chromosome 3q. Am J Hum Genet 60:296–304

Wirtz MK, Samples JR, Rust K, Lie J, Nordling L, Schilling K, Acott TS, Kramer PL (1999) GLC1F, a new primary open-angle glaucoma locus, maps to 7q35-q36. Arch Ophthalmol 117:237–241